development may be completed on the tree or after falling. Apparently no other author has described full development of the embryo on the tree and Hirase's statement is probably in error.) Seward and Gowan (1900), in their memoire, apparently following Hirase, state that fertilization may occur before or after ovule fall. Strangely, neither Coulter and Chamberlain's textbook (1917) nor Chamberlain's much later text (1935) mentions place of fertilization. Chadefaud (1944) found that in Paris "ovules fall at the time of fertilization but also afterward." Studies made by the writer some years ago showed that in Ithaca, N. Y., fertilization may occur in late September in green ovules on the tree; in October in ripe ovules on the tree, or on the ground, even some days after they have fallen. The time of fertilization varied considerably in ovules on the same tree and among different trees in the same season and on the same tree in different seasons. Favre-Duchartre (1943) reported that fertilization may occur either just before, or after ovule fall, and that embryo development may continue to maturity even in the laboratory. Dangeard (1946) reported finding embryos in ovules on trees in mid-September in one year and in mid-August in another. Martens (1951) surmised that time of fertilization is climatically controlled. In the light of the writer's experience it seems more likely that it is related to age and time of pollination of the individual ovule. Fertilization in *Ginkgo* clearly may occur over a fairly long period and either on the tree or on the ground. The existence of this condition in a representative of a primitive seed-plant is important because it is probably an illustration of the ancient step in seed evolution when fertilization was transferred from the ground to the mother sporophyte. The survival of *Ginkgo* is, in one more way, most fortunate — as a demonstration of a critical step in the phylogenetic history of the seed.

Emberger's classification breaks down on the basis that *Ginkgo* falls in both of his groups. Further, Florin (1950) points out that Emberger's classification separates the Cordaitinae and Ginkgoinae from the Coniferae. And the growing opinion, substantiated by evidence from several fields, is that the Cordaitales, Ginkgoales, Coniferales, and Ephedrales form a natural group (Eames 1953).

DORMANCY IN PRIMITIVE SEEDS

Ginkgo, together with the cycads, illustrates another important step in the development of the seed, a step in the establishment of dormancy in the embryo. Though dormancy is often considered an essential character of the seed, the story of its development has received little attention. Dormancy of the embryo is an outstanding feature of the seeds of angiosperms and higher gymnosperms. The presence or absence of dormancy in the lower living gymnosperms — *Ginkgo* and the cycads — is rarely mentioned. In the lower fossil gymnosperms — pteridosperms and cordaites — the condition is unknown. Florin (1950) remarks "It appears probable that the ovules of the ancient gymnosperms always had a rest period, either after fertilization or after the shedding stage." He has also said

that the common absence of embryos in paleozoic seeds may well be explained "by the shedding of ovules rather than seeds." Arnold (1948) believes that paleozoic ovules were shed at a particular stage of maturity and that they entered a rest period at that time. (Arnold also suggests that the presence of a hard, durable integument enclosing the gametophyte may be evidence of dormancy in the ovules. But this condition is not necessarily evidence of a resting stage, for *Ginkgo* and some of the conifers, especially species of *Podocarpus*, have such histologically hard and tight layers before fertilization.) The writer agrees with Florin and Arnold that the ancient "seeds" without ovules are unfertilized ovules or seeds with early stages of embryos and that the absence of embryos suggests a resting period at the time of shedding or soon thereafter. Though *Ginkgo*, in its shedding of ovules and seeds with proembryos, shows in living plants the condition suggested for paleozoic plants, it does not give support to the existence of a rest period at this stage.

Neither *Ginkgo* nor the cycads show fixed dormancy at any stage; they do show induced dormancy, a dormancy induced by conditions unfavorable to continuous growth of the embryo or to germination if the embryo is mature. Such induced dormancy may well be a step to the fixed dormancy of other plants.

For *Ginkgo*, published statements imply embryo growth continuing until germination. Van Tieghem (1884) found embryo development through the winter. Hirase (1894) reports that the fall of the seed does not interrupt the growth of the embryo which continues until spring. Chamberlain (1935) states that embryo development continues in the laboratory or under stratification out-of-doors to germination in April; there is little or no dormancy, but development is more rapid in the laboratory. The writer followed embryo development in stratified seeds kept out-of-doors through the winter. (The seeds used were from a lot that had fallen and in which fertilization was occurring on the ground.) The studies showed that growth continued throughout the winter whenever temperatures were above freezing, and germination occurred in April without any apparent dormancy. Seeds held in warm places probably do complete embryonic growth and lie dormant until conditions are favorable for germination; here dormancy is induced.

In the cycads also, embryo growth is often stated or implied as continuing after the seed is shed but direct statements concerning dormancy are rare. Baird (1939) states that in *Macrozamia reidlei* growth continues in the seed for over 12 months without cessation before germination. The author has noted a similar condition in *Cycas circinalis*, with embryo development through several months. Chamberlain (1910), describing the embryogeny of *Dioon edule*, states that in this plant germination occurs without a dormant period but that seeds germinated after lying in a laboratory for over two years. Chamberlain later (1919) wrote "the cycad has no resting period, development being continuous from fertilization to old age and death."

Doubtless in both the cycads and *Ginkgo*, embryo development is con-

tinuous until germination under favorable climatic conditions, but dormancy may develop under conditions unfavorable to germination. Dormancy is not fixed but may be induced. These plants provide a step between continuous embryo-seedling development and the establishment of a dormant period at a determined stage in embryo development. In definition of the seed, no line can be drawn between the absence and the presence of dormancy in the embryo. It may be desirable — it is certainly convenient — to draw a line between the ovule and the seed, the presence of an embryo of any stage determining a seed. But it is a bit awkward to say that the *Ginkgo* tree sheds both ovules and seeds — that some of the fruit-like structures are ovules and others seeds without being able to point out which are which.

Summary. The definition of "seed" is discussed and the bearing of *Ginkgo* and its "seeds" on Emberger's segregation of Prephanerogams from Phanerogams. *Ginkgo*, in that fertilization takes place either on the tree or on the ground, falls in both of these groups, showing the classification to be artificial. This genus shows the important step, in the evolution of the seed, of the transfer of fertilization from the ground to the mother sporophyte. With the cycads it shows a step in the establishment of dormancy of the embryo in the seed.

The writer wishes to acknowledge his indebtedness to Florin and Martens on whose papers he has drawn freely in this discussion.

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DEPT. OF BOTANY,
CORNELL UNIVERSITY.

SOME FACTORS IN POLLEN GERMINATION CALCIUM SALTS, DEXTROSE, DRYING

ANNA F. FAULL

IN HONOR OF IRVING W. BAILEY whose pioneer studies in cell structure, behavior and organization have been an inspiration, this paper is presented. It is a brief summary and discussion of experiments reported at the last three meetings of the A. I. B. S. but otherwise unpublished.

Pollen is notorious for the uncertainty of its behavior in germination and tube growth. Yet very little work has been done on the factors involved. A few studies have been made as part of more comprehensive programs. Bungenberg de Jong and Henneman (1934) in their work on cell models and neutral salts reported on pollen. Smith (1939) tested indole-3-acetic acid as a pollen germinant. Loo and Hwang (1944) included pollen in a series of papers on indole-3-acetic acid, manganese sulfate and colchicine as growth stimulants. Thomas, Gauch and Dugger, Jr. (1952) extended work on boron effects to include pollen, and O'Kelley (1954) used C-14 sugars to examine the rôle of carbohydrates in pollen growth. A brief summary of the literature has been made by Nitsch (1953) in his review of fruit growth.

In the present study of pollen physiology suggested by K. V. Thimann [1] in 1945, it soon became apparent that information on pollen germination requirements was incomplete. The essential tools of a reliable germinating technique and a growth medium were still lacking. To develop these it was necessary to determine the factors involved in pollen growth and their operation in terms of cell structure.

A preliminary survey of chemical stimulants and environmental factors was made in March to June, 1946–1949, on 28 species of the Texas Gulf Coast. None germinated satisfactorily in water, but, used separately or together, calcium salts and dextrose sometimes stimulated both germination and tube growth. Potassium, nitrogen, phosphorus, manganese and carbonate ions, indole-3-acetic acid, acetic acid and ammonia were ineffective although potassium as KOH or K_2CO_3 produced pronounced swelling of the intine wall.[2] Of the environmental factors the significant ones appeared to be humidity and age rather than temperature, light or acidity.

Intensive work on calcium and the related strontium and barium salts was done in Texas in the spring of 1950 and that of 1951. *Crinum* was the primary plant material. In 1953 a similar study of the effect of drying

[1] The author wishes to acknowledge Dr. Thimann's interest and kindness in suggesting pollen as plant material.

[2] In M/40–80 KOH or under mechanical pressure in weaker solutions of either KOH or K_2CO_3 swelling of the intine was followed by rupture of the extine wall and escape of the entire cell with the intine wall intact. Emerged cells were enormously enlarged with a thin wall.

was made on pollen of *Crinum*, *Nothoscordum* and *Hymenocallis*. Germination in dextrose and other sugar solutions was studied in Cambridge, Mass., June–September 1954, using pollen from a number of Irid, Amaryllid and Liliaceous genera.

Experimental technique was simple. Equipment consisted of a compound microscope with ocular micrometer, hollow-ground glass slides and covers for germinating chambers, a gold prospector's scale (accurate to 1 mg. ±), C.P. chemicals, procelain-distilled water, pipettes graduated to 1/10 ml. and a 100 cc. cylinder. Pollen for each experiment was taken from a single flower or anther from which it was dusted onto single drops of the solutions. All chemicals were used in concentration series with one or more controls in water and dextrose or calcium. Stock solutions (M/5. M, 4M or saturated) were kept at 9° C±. Series were made by progressively diluting the stock one half: dilutions of one tenth were less satisfactory. To obtain progressive stages of drying, anthers from a single flower were exposed to room conditions over a period of days or weeks often with one or more held for comparison in a saturated atmosphere.

For all experiments % germination, tube length and elapsed time were recorded at the end of two or more hours. Tube growth might continue longer but most germination had occurred by the end of two hours and there was no discernible change in germination figures after four hours.

Results of the experiments may be summarized briefly.

Calcium (*at. wt. 40*). — Calcium salts were used alone and in combination with M/40 dextrose. Pollen for the 1950 tests showed no germination in water or in M/40 dextrose; 1951 material had O – 44% germination in water, O – 91% in M/40 dextrose.

All of the calcium salts tested — $Ca(NO_3)_2$, Ca Ac, $CaCO_3$, $Ca(H_2PO_4)_2$ — were effective alone in aqueous solution. They initiated germination in pollen of *Crinum* with none in water, dextrose or other solutions tested; they increased the percentage for pollen with some germination in water; they stimulated tube growth at least in the early stages.

Pollen response was dependent upon the concentration of calcium (FIGS. 1 + 2). Dilute solutions only were effective in varying degrees over a broad range (M/20 – M/40960±). Effective ranges for the different salts were: $Ca(NO_3)_2$ M/20 to M/5120–40960, Ca Ac M/20 to M/2560–40960, $CaCO_3$ sat. to M/5120, $Ca(H_2PO_4)_2$ sat. to M/2,500,000 – 10,000,000.[3] All ranges showed a well-defined optimum and often a single optimum concentration. Characteristic optima for the 1950–51 material usually included several concentrations in the M/20–M/2560 range with a single optimum between M/160 and M/640 [$Ca(H_2PO_4)_2$ sat.–M/650,000 usually with no single optimum concentration].

Optima and the dilute limit of the total range varied. They were

[3] Concentrations of the almost insoluble calcium carbonate and calcium acid phosphate were calculated from the amount of salt dissolving in 50cc. distilled water in two weeks. Since degree of solubility varies with the amount of CO_2 present and other factors, figures for these salts must be considered approximate.